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Confirmation No.	:	5046
Applicants	:	Yuzhang Wu et al.
Filing Date	:	02/15/2006
Art Unit	:	1648
Examiner	:	PENG, BO
Title of invention	:	IMMUNOGEN FOR PREPARATION OF THERAPEUTIC VACCINES OR DRUGS FOR TREATMENT OF HEPATITIS B AND THE PRODUCING METHOD AND USE THEREOF

Commissioner for Patents
U.S. Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CRF § 1.132

Sir:

I, Yuzhang Wu, declare as follows:

1. I hold a Ph.D. in viral biology from Third Military Medical University. Attached please find my curriculum vitae. I presently hold the position of Director of Institute of Immunology, PLA, National Engineering Lab for Peptides Drugs, National Center for Immunoproducts Engineering and Technology Reserch, and National Center of Bioindustry Scale-up Unit. I am the first named inventor of the above-identified patent application and are familiar with the claimed immunogens.

2. I have reviewed the Office Action dated September 2, 2010, including the positions taken by the PTO with respect to several references. I have also particularly reviewed the references US Patent No.6,322,789(Hereinafter "Vitiello") and Tam et al., Vaccine engineering: Enhancement of immunogenicity of synthetic peptide vaccines related to hepatitis in chemically defined models consisting of T- and B-cell epitopes, Proc. Natl. Acad. Sci. U.S.A. 86:9084-9088 (1989) (Hereinafter "Tam").

3. The claimed invention relates to a polypeptide immunogen comprising sequence 1, sequence 2 and sequence 3 which are a Th cell epitope, a CTL epitope and a B cell epitope, respectively. During the prosecution of the present application, a polypeptide immunogen comprising a T helper epitope of SEQ ID NO:6, a CTL epitope of SEQ ID NO:23 and a B cell epitope of SEQ ID NO:48 was elected.

The Vitiello reference teaches a polypeptide immunogen comprising a T helper epitope (SEQ ID NO:6 of the present application) and a CTL epitope (SEQ ID NO:23 of the present application) from HBV, but is silent as to incorporating a B cell epitope (SEQ ID NO:48 of the present application) into the polypeptide.

The Tam reference teaches a synthetic peptide immunogen comprising (1) the a determinant of the S region (S protein) of HBV surface antigen and (2) residues 12-26 of the pre-S(2) region of the middle protein, which comprises SEQ ID NO:48 of the present application.

The Examiner alleges that a polypeptide immunogen comprising a T helper epitope of SEQ ID NO:6, a CTL epitope of SEQ ID NO:23 and a B cell epitope of SEQ ID NO:48 of the present application is obvious over the combination of the Vitiello reference and the Tam reference.

4. I disagree with the Examiner's conclusion. One of the key issues in current immunology is how an immune protection is generated. Up to now, the only clue is that a protective immune requires a set of epitopes. However, as to how many epitopes, how many kinds of epitopes and how to combine and match them in order to generate a protective immune, little is known. Thus, one cannot simply combine one epitope and another different epitope chosen from numerous reported epitopes and assert that the combination of the two epitopes is obvious. Naturally, the combination of 3 epitopes of the present invention cannot be viewed as a simple combination of the Vitiello reference and the Tam reference. In the present invention, we have investigated combinations of 1 to 5 epitopes, and found that a combination of 2 epitopes was better than 1 epitope, a combination of 3 epitopes was significantly and surprisingly better than a combination of 2 epitopes, and the combinations of 4, 5, and 3 epitopes were not significantly different from each other. Our finding suggests that 3 epitopes were the optimal epitope set for inducing a complete immune response according to the present invention.

5. The significant difference between effects of an immunogen comprising 3 epitopes and an immunogen comprising 2 epitopes were illustrated below.

In the following experiments, three immunogens were compared and the structures of the immunogens were as follows.

Immunogen D1 is an immunogen of the present invention, comprising a Th cell epitope, a CTL epitope and a B cell epitope (which were sequence 1, sequence 2, and sequence 3, respectively), and has the structure of $CH_3(CH_2)_{-14}CO$ KSS QYIKANSKFIGITE AAA FLPSDFFPSV GGG DPRVRGLYFPA. The sequence 1, sequence 2, and sequence 3 have the amino acid sequences of QYIKANSKFIGITE, FLPSDFFPSV, and DPRVRGLYFPA, respectively.

Immunogen D2 is a reference immunogen comprising only two epitopes, Th epitope+CTL epitope (namely, sequence 1+sequence 2), and has the structure of $CH_3(CH_2)_{-14}CO$ KSS QYIKANSKFIGITE AAA FLPSDFFPSV.

Immunogen D3 is a reference immunogen comprising only two epitopes, Th epitope+B epitope (namely, sequence 1+sequence 3) and has the structure of $CH_3(CH_2)_{-14}CO$ KSS QYIKANSKFIGITE GGG DPRVRGLYFPA.

6. Example A: Activity of Immunogen D1 to induce CTL was surprisingly

higher as compared to the additive activity of Immunogens D2 and D3.

Experiments as described in Example 55 (see paragraph [0191]) of the application were conducted to test the generation of HBV-specific effective CTL from hepatitis patients' PBMC and cytotoxicity induced by immunogens D1, D2 and D3, respectively.

Acute and chronic hepatitis blank control group, acute hepatitis subject group administered with a test drug, and chronic hepatitis subject group administered with a test drug were included. The PBMCs from the patients were cultured *in vitro*, and stimulated repetitively to generate antigen-specific CTL cells and amplified the number thereof. Then, they were used to conduct cell toxicity experiments on HepG2.2.15 cells. The results for the proportion of target cell-specific lysis rate (%) were compared and shown below:

Test drug	Proportion of target cell-specific lysis rate (%)	
	PBMC from acute hepatitis patient in convalescent phase	PBMC from chronic hepatitis patient
Blank control	3±5.1	3±2.8
D1	68.6±5.3	42.6±3.4
D2	21.3±6.3	15.1±5.2
D3	5±3.1	4±3.5

It could be seen that the activity of Immunogen D1 to induce CTL was surprisingly increased as compared to the additive activity of Immunogens D2 and D3.

7. Example B: Activity of Immunogen D1 to induce interferon-secreting cells was significantly and surprisingly increased as compared to the additive activity of Immunogens D2 and D3.

The activities of D1, D2, D3 to induce CTL in HBV transgenic mice were compared using experiments as described in Example 58 (see paragraph [0194]) of the present application. Specifically, HBV-DNA transgenic mice (ayw type Kunming species mice transfected with the full length gene of HBV(1.3kb)) were used. The animals were grouped randomly, with 15 mice per group. The mice were administered respectively with 3 doses, 5 ug, 10 ug and 100 ug/mouse, subcutaneously below both costal regions and at both postpede palms. Booster was given once per week for 3 times. IFN-a2b (15000U/mouse)was included as positive control, and physiological saline was included as negative control. On the 30th day after the end of drug administration, the spleen of the mice was taken and the spleen lymphocytes were isolated. The lymphocyte cells were stimulated *in vitro* with 10ng/ml of the test drug for 3 days. The expression frequency of IFN-? secreting cells in the periphery blood lymphocytes was measured using ELI-SPOT assay. The result showed that on the 30th day after the end of immunization, the expression frequency of IFN-? secreting cells in the periphery blood lymphocytes increased with the increase of the immunization dose. The increase in the Immunogen D1 group was most evident, and the frequency of

IFN-? secreting cells in the peripheral blood lymphocytes with 5 ug/mouse, 30 days *in vivo* induction clearly increased, wherein the highest frequency detected was 3660±112 IFN-? secreting cells/10^6PBMC, which was 200 times that of the Immunogen D2 group, and 1000 times that of Immunogen D3 group, as shown in the Table below:

Table. Frequency of IFN-? secreting cells in the peripheral blood lymphocytes with 5 ug/mouse, 30 days *in vivo* induction

Test drug	IFN-? secreting cells/10^6 PBMC
D1	3660±112
D2	183±45
D3	3±7
IFN-a2b (15000U/mouse)	75±11

It could be seen that activity of Immunogen D1 to induce interferon-secreting cells was significantly and surprisingly increased as compared to the additive activity of Immunogens D2 and D3.

8. Example C: Immunogen D1 leaded to significant decrease of HBV surface antigen amount in contrast to no significant change by Immunogens D2 and D3.

Experiments as described in Example 59 (see paragraph [0195]) of the present application were conducted to determine the inhibition of HBV surface antigen in HBV transgenic mice by Immunogens D1, D2 and D3, respectively. Specifically, HBV-DNA transgenic mice (ayw type Kunming species mice transfected with the full length gene of HBV (1.3kb)) were used. The animals were grouped randomly, with 15 mice per group. The mice were administered respectively with 3 doses, 10, 100 and 1000 U/mouse, subcutaneously below both costal regions and at both postpede palms. Booster was given once per week for 3 times. IFN-a2b (15000U/mouse) was included as positive control, and physiological saline was included as negative control. On the 10^{th}, 20^{th}, and 30^{th} days after the end of the three immmunizations, the blood were taken from the mice, and the serum were separated. The amounts of HBsAg in the serums were measured using ELISA assay respectively. The results showed that 30 days after the end of immunization, in the mice of Immunogen D1 treatment group, the serum HBsAg amount significantly decreased by more than 200 times in a manner of dose-dependence and time-dependence, whereas the serum HBsAg amount in the Immunogen D2 treatment group or Immunogen D1 treatment group did not change significantly.

It could be seen that the activity of Immunogen D1 to decrease HBV surface antigen amount was significantly higher than the additive activity of Immunogens D2 and D3.

9. Example D: Activity of Immunogen D1 to inhibit HBV virus replication was significantly enhanced as compared to the additive activity of Immunogens D2 and D3

Inhibition of virus replication in HBV transgenic mice were measured as described in Example 60 (see paragraph [0196]) of the present application. Specifically, HBV-DNA transgenic mice (ayw type Kunming species mice transfected with the full length gene of HBV (1.3kb)) were used. The animals were grouped randomly, with 15 mice per group. The mice were administered respectively with 3 doses, 10, 100 and 1000 U/mouse, subcutaneously below both costal regions and at both postpede palms. Booster was given once per week for 3 times. IFN-a2b (15000U/mouse) was included as positive control, and physiological saline was included as negative control. On the 10^{th}, 20^{th}, and 30^{th} days after the end of the three immmunizations, the blood were taken from the mice, and the serum were separated. The HBV DNA copy numbers in the serums were detected using quantitative PCR analysis respectively. The results showed that for Immunogen D1 treatment group, on the 20^{th} and 30^{th} days, the serum HBV DNA copy number was significantly reduced in a manner of dose-dependence and time-dependence, wherein all of them were $<10^2$ copies/ml on the 30^{th} day; for Immunogen D2 treatment group, on the 30^{th} day, the serum HBV DNA copy number was evidently decreased, with 10^4~10^3 copies/ml in 12/15 mice and no significant change in 3/15 mice; and for Immunogen D3 treatment group, there was no clear change.

It could be seen that the activity of Immunogen D1 to inhibit HBV virus replication was significantly enhanced as compared to the additive activity of Immunogens D2 and D3.

10. Example E: Immunogen D1 caused strong CTL response, reduction of virus amount and serological transformation in the tested human population.

Immunogen D1 was authorized by the SFDA to enter the clinical phase. The II(a) stage clinical tests showed that, in 10 subjects of the 900ug treatment group (3 subcutaneous injections, at 6 week intervals), 1 week after the three injections, 10/10 subjects generated strong CTL response, the frequency of CTL in PBMC was increased from 0.2±0.3% before the injection to 5-7%. In the 84^{th} week, in 7/10 subjects, the serum HBV DNA copy number was significantly decreased by more than 2log; in 5/10 subjects, the serum HBV DNA copy number was $<10^2$ copies/ml; in 5/10 subjects, the serum showed HBeAg/HBeAb serological transformation and negative HbeAg. Immunogens D2 and D3 were not authorized by the SFDA to enter into clinical phase.

11. Even if one were motivated to combine a Th cell epitope, a CTL epitope and a B cell epitope to generate an immunogen, an ordinary skilled artisan would have expected that Immunogen D1 comprising a Th cell epitope, a CTL epitope and a B cell epitope would have effect which was not more than the additive effects of Immunogen D2 comprising only Th epitope and CTL epitope and Immunogen D3 comprising only Th epitope and B epitope. Therefore, there was no expectation that Immunogen D1 would generate greater effect than the sum of the effect of Immunogen D2 and the effect Immunogen D3 (namely, the additive effect of Immunogens D2 and D3).

From the results of the above Examples A-E, however, it can be seen that the effects of Immunogen D1 are significantly much higher than the additive effects of Immunogens D2 and D3.

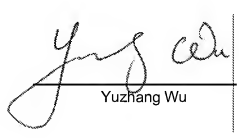
Accordingly, it can be concluded that Immunogen D1 can generate

unexpectedly better effects as compared to Immunogens D2 and D3, and that an immunogen of the present invention comprising a Th cell epitope, a CTL epitope and a B cell epitope is not obvious at the time of invention.

12. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like, so made, are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted

February 25, 2011
Date



Yuzhang Wu

Curriculum Vitae

Yuzhang Wu, MD, PhD

Professor, Director
Institute of Immunology, PLA
Third Military Medical University
Chongqing, 400038, P.R. China
Tel: 86-23-68752680
Fax: 86-23-68772348
e-mail: wuyuzhang@yahoo.com

Education

PhD, Third Military Medical University, 1993
MSc, Third Military Medical University, 1989
MD, Second Military Medical University, 1984

Experience and Appointment

2000-	Director
	Institute of Immunology, PLA
	National Engineering Lab for Peptides Drugs
	National Center for Immunoproducts Engineering and Technology Reserch
	National Center of Bioindustry Scale-up Unit
1996-2000	Professor
	Institute of Immunology, PLA
	Third Military Medical University
	Chongqing, China
1994-1996	Associate Professor
	Institute of Immunology, PLA
	Third Military Medical University
	Chongqing, China
1985-1994	Assistant Professor
	Department of Epidemiology, PLA
	Third Military Medical University
	Chongqing, China

Scientific Rewards

1996 Top 100 Outstanding Young Scientists in Medical Science, China
New Star in Scientific Research, PLA
2000 Hundreds Talented Scientists in the New Century, China
2002 Outstanding Youth Foundation ,National Science Foundation of China
Silver Star in Scientific Research, PLA
2005 Natural Science Prize, Level I, Chongqing

2006 Changjiang Scholar
Annual 10 Research Achievements in Science and Technology, China
2008 Golden Star in Scientific Research, PLA
Natural Science Prize, Level II, Ministry of Education, China
2009 Medical Science Achievement Prize, Level I, Chinese Medical Society

Recent Research Projects

No.	Grant No.	Grants	Foundation	Fund ($x10^4$Yuan)	Principal
1.	2009ZX09503	Critical technology research on peptide biological products manufacturing and modification	Key Project of National Science and Technology, China	750.0	Yuzhng Wu
2.	2008ZX10202	Clinical research on the therapeutic vaccine of viral hepatitis B	Key Project of National Science and Technology, China	1500.0	Yuzhng Wu
3.	——	Pilot production center of national biotechnology industry base	National Development and Reform Commission ,China	1200.0	Yuzhng Wu
4.	——	National engineering research center for biological products	National Science and Technology Ministry, China	1200.0	Yuzhng Wu
5.	2006AA02A207	Development of viral hepatitis B therapeutic vaccine	National Project for High Technology	898.0	Yuzhng Wu
6.	2007CB512401	Immune recognition of autoantigen and design of negative vaccine	The National Principal Program for Basic Research, China	350.0	Yuzhng Wu
7.	30930086	Cellular and molecular mechanism of immune injury in the infection of Hepatitis B virus	The Key Project of the National Natural Science Foundation of China	185.0	Yuzhng Wu
8.	30490241	Immune recognization based on epitope structure	The Principal Program of the National Natural Science Foundation of China	170.0	Yuzhng Wu
9.	——	Changjiang Scholar Award	Changjiang Scholar Award Foundation	200.0	Yuzhng Wu
10.	CSTC2005CB5006	Center for engineering research of peptide therapeutics, Chongqing	Science and Technology Innovation Foundation, Chongqing, China	100.0	Yuzhng Wu
11.		Molecular and cellular Immunologic mechanism of hepatic injury and protection in HBV infection	National Natural Science Foundation of China	185. 0	Yuzhang wu

Publications:

1. Zou L,Zhang J,Li J,Liu N,Chai L,Li N,Liu T, Li L, Xie Zh, Liu H, Wan Y, ***Wu Y***. The GTPase Rab3b/3c-positive recycling vesicles are involved in cross-presentation in dendritic cells. ***Proc Natl Acad Sci U S A.*** 2009 Aug 28. [Epub ahead of print]
2. Yang D,Wang H,Ni B,He Y,Li J,Tang Y, Fu X,Wang Q,Xu G,Li K,Yang Z,***Wu Y***. Mutual activation of CD4+ T cells and monocytes mediated by NKG2D-MIC interaction requires IFN-gamma production in systemic lupus erythematosus. ***Mol Immunol.*** 2009 Apr;46(7):1432-42.
3. Wang H, Yang Z, Li F, He Y, Wang S, Yang D, ***Wu Y***. Insertion of a targeting peptide on

capsid surface loops of human papillomavirus type-16 virus-like particles mediate elimination of anti-dsDNA Abs-producing B cells with high efficiency. ***J Immunother.*** 2009 Jan;32(1):36-41

4. Tang Y, Chen Y, Ni B, Yang D, Guo S, ***Wu Y***. Up-regulation of the expression of costimulatory molecule CD40 in hepatocytes by hepatitis B virus X antigen. ***Biochem Biophys Res Commun.*** 2009 Jun 19;384(1):12-7.
5. Chen Z, He W, Liang Z, Yan P, He H, Tang Y, Zhang J, Shen Z, Ni B, ***Wu Y***. Li J. Protein prime-peptide boost as a new strategy induced an Eppin dominant B-cell epitope specific immune response and suppressed fertility. ***Vaccine.*** 2009 Jan 29;27(5):733-40.
6. Chen Y, Zhang J, Guo G, Ruan Z, Jiang M, Wu S, Guo S, Fei L, Tang Y, Yang C, Jia Z, ***Wu Y***. Induced B7-H1 expression on human renal tubular epithelial cells by the sublytic terminal complement complex C5b-9. ***Mol Immunol.*** 2009Jan;46(3):375-83.
7. Li F, Yang D, Wang Y, Liu B, Deng Y, Wang L, Shang X, Tong W, Ni B, ***Wu Y***. Identification and modification of an HLA-A*0201-restricted cytotoxic T lymphocyte epitope from Ran antigen. ***Cancer Immunol Immunother.*** 2009 May 9. [Epub ahead of print]
8. Zhang J, Zou L, Liu Q, Li J, Zhou J, Wang Y, Li N, Liu T, Wei H, Wu M, Wan Y, ***Wu Y***. Rapid generation of dendritic cell specific transgenic mice by lentiviral vectors. ***Transgenic Res.*** 2009 May 26. [Epub ahead of print]
9. Luo N, Wu Y, Chen Y, Yang Z, Guo S, Fei L, Zhou D, Yang C, Wu S, Ni B, Hao F, ***Wu Y***. Upregulated BclG(L) expression enhances apoptosis of peripheral blood CD4(+) T lymphocytes in patients with systemic lupus erythematosus. ***Clin Immunol.*** 2009 Jun 11. [Epub ahead of print]
10. Shang X, Wang L, Niu W, Meng G, Fu X, Ni B, Lin Z, Yang Z, Chen X, ***Wu Y***. Rational Optimization of Tumor Epitopes Using In Silico Analysis Assisted Substitution of TCR Contact Residues. ***Eur J Immunol.*** 2009 Jul 10. [Epub ahead of print]
11. Ruan Z, Yang Z, Wang Y, Wang H, Chen Y, Shang X, Yang C, Guo S, Han J, Liang H, ***Wu Y***. DNA vaccine against tumor endothelial marker 8 inhibits tumor angiogenesis and growth. ***J Immunother.*** 2009 Jun;32(5):486-91.
12. Mou Z, He Y, ***Wu Y***. Immunoproteomics to identify tumor-associated antigens eliciting humoral response. ***Cancer Lett.*** 2009 Jun 18;278(2):123-9. Epub 2008 Oct 17. Review.
13. Li H, Zou Q, Xie Z, Liu Y, Zhong B, Yang S, Zheng P, Yang F, Fang Y, ***Wu Y.*** A haplotype in STAT4 gene associated with rheumatoid arthritis in Caucasians is not associated in the Han Chinese population, but with the presence of rheumatoid factor. ***Rheumatology (Oxford).*** 2009 Sep 8. [Epub ahead of print]
14. Li J, Wang Y, Liang Y, Ni B, Wan Y, Liao Z, Chan KH, Yuen KY, Fu X, Shang X, Wang S, Yi D, Guo B, Di B, Wang M, Che X, ***Wu Y***. Fine antigenic variation within H5N1 influenza virus hemagglutinin's antigenic sites defined by yeast cell surface display. ***Eur J Immunol.*** 2009 Oct 1. [Epub ahead of print]

15. Wei J, Li J, Zhang X, Tang Y, Wang J, ***Wu Y***. A naturally processed epitope on rotavirus VP7 glycoprotein recognized by HLA-A2.1-restricted cytotoxic CD8+ T cells. ***Viral Immunol.*** 2009 Jun;22(3):189-94

16. Yan P, He W, Liang Z, Chen Z, Shang X, He H, Tang Y, Ni B, Zhang J, Shen Z, ***Wu Y***, Li J. A novel dominant B-cell epitope of FSHR identified by molecular docking induced specific immune response and suppressed fertility. ***Gynecol Endocrinol.***2009 Jun 23:1-11.

17. He Y, Mou Z, Li W, Liu B, Fu T, Zhao S, Xiang D, ***Wu Y***. Identification of lMPDH2 as a tumor-associated antigen in colorectal cancer using immunoproteomics analysis. ***Int J Colorectal Dis.*** 2009 Jul 14. [Epub ahead of print]

18. Liu D, Zou L, Li W, Wang L, ***Wu Y***. High-level expression and large-scale preparation of soluble HBx antigen from Escherichia coli. ***Biotechnol Appl Biochem.*** 2009 Jul 16. [Epub ahead of print]

19. Liu K, Yang X, ***Wu Y***, Li J. Rotavirus Strategies to evade host antiviral innate immunity. ***Immunol Lett.*** 2009 Aug 24. [Epub ahead of print]

20. Xie Z, Chen Y, Zhao S, Yang Z, Yao X, Guo S, Yang C, Fei L, Zeng X, Ni B, ***Wu Y***. Intrahepatic PD-1/PD-L1 up-regulation closely correlates with inflammation and virus replication in patients with chronic HBV infection. ***Immunol Invest.*** 2009;38(7):624-38.

21. Wang Y, Bai J, Li F, Wang H, Fu X, Zhao T, Xu W, Zhang J, Ni B, ***Wu Y***. Characteristics of expanded CD4+CD28null T cells in patients with chronic hepatitis B. ***Immunol Invest.*** 2009; 38 (5):434-46.

22. Wang H, Ruan Z, Wang Y, Han J, Fu X, Zhao T, Yang D, Xu W, Yang Z, Wang L, Chen Y, ***Wu Y***. MHC class I chain-related molecules induced on monocytes by IFN-? promote NK cell activation. ***Mol Immunol.*** 2008 Mar; 45(6): 1548-56

23. Ruan Z, Wang H, Ren Y, Chen Y, Han J, Pang X, Liang H, ***Wu Y***. Pseudo receptor probes: A novel pseudo receptor-based QSAR method and application into studies on a new kind of selective vascular endothelial growth factor-2 receptor inhibitors. ***Chemometr Intell Lab.*** 2008 July; 92(2)157-168

24. Yang Z, Wang L, Niu W, ***Wu Y***, Zhang J, Meng G. Increased $CD4^{+}CD25^{+}FoxP3^{+}$ regulatory T cells in the blood of nonresponders after standard hepatitis B surface antigen vaccine immunization. ***Clin Immunol.*** 2008 May; 127(2): 265-6.

25. He Y, Mao L, Lin Z, Deng Y, Tang Y, Jiang M, Li W, Jia Z, Wang J, Ni B, ***Wu Y***. Identification of a common HLA-A*0201-restricted epitope among SSX family members by mimicking altered peptide ligands strategy. Mol Immunol. 2008 May; 45(9): 2455-64.

26. Yang Z, Wang L, Wang H, Shang X, Niu W, Li J, ***Wu Y***. A novel mimovirus vaccine containing survivin epitope with adjuvant IL-15 induces long-lasting cellular immunity and high antitumor efficiency. ***Mol Immunol.*** 2008 Mar; 45(6): 1674-81.

27. Guo B, Ma ZW, Li H, Xu GL, Zheng P, Zhu B, ***Wu YZ***, Zou Q. Mapping of binding epitopes of a human decay-accelerating factor monoclonal antibody capable of enhancing

rituximab-mediated complement-dependent cytotoxicity. ***Clin Immunol.*** 2008 Aug; 128(2): 155-63.

28. Wang H, Yang D, Xu W, Wang Y, Ruan Z, Zhao T, Han J, ***Wu Y***. Tumor-derived soluble MICs impair CD3+CD56+ NKT-like cell cytotoxicity in cancer patients. ***Immunol Lett.*** 2008 Sep 30;120(1-2):65-71
29. Lin ZH, Long HX, Bo Z, Wang YQ, ***Wu YZ***. New descriptors of amino acids and their application to peptide QSAR study. ***Peptides*** 2008 Oct; 29(10):1798-805.
30. Yang Z, Wang S, Zhang J, Wang L, ***Wu Y***. Expression of PD-1 is up-regulated in CD4(+)CD25(+) FoxP3(+) regulatory T cell of non-responders after hepatitis B surface antigen vaccine immunization. ***Clin Immunol.*** 2008 Oct; 129(1): 176-177
31. Xia R, Hao F, Mou Z, ***Wu Y***. Identification of proteins involved in aggregation of human dermal papilla cells by proteomics. ***J. dermatol. sci***. 2007, 48(3):189-197
32. Chen Y, Yang C, Jin N, Xie Z, Fei L, Jia Z, ***Wu Y***. Sinomenine promotes differentiation but impedes maturation and co-stimulatory molecule expression of human monocyte-derived dendritic cells. ***Int Immunopharmacol***. 2007 Aug;7(8):1102-1110.
33. Xu DH, Zhou CH, Xia YP, Qiu ZY, ***Wu YZ***, Jia ZC, Zhou W. Cytotoxic T lymphocyte response induced by an improved synthetic lipopeptide vaccine against cervical cancer. ***Acta Pharmacol Sin.*** 2007 May;28(5):695-702.
34. Zhao G, Ni B, Jiang H, Luo D, Pacal M, Zhou L, Zhang L, Xing L, Zhang L, Jia Z, Lin Z, Wang L, Li J, Liang Y, Shi X, Zhao T, Zhou L, ***Wu Y***, Wang X. Inhibition of severe acute respiratory syndrome-associated coronavirus infection by equine neutralizing antibody in golden Syrian hamsters. ***Viral Immunol***. 2007 Spring;20(1):197-205.
35. Zhou L, Ni B, Luo D, Zhao G, Jia Z, Zhang L, Lin Z, Wang L, Zhang S, Xing L, Li J, Liang Y, Shi X, Zhao T, Zhou L, ***Wu Y***, Wang X. Inhibition of infection caused by severe acute respiratory syndrome-associated coronavirus by equine neutralizing antibody in aged mice. ***Int Immunopharmacol***. 2007 Mar; 7(3):392-400.
36. Ni B,Zhou J,Dong Y,Peng J,Wu X,Li R,Chen M,Zhou C,Tan Y, ***Wu Y***. Interleukin-1 up-regulates the expression and activity of 26S proteasome in burned rat. ***Burns***.2007 Aug; 33(5):621-7.
37. He YJ, Wu YZ, Mou ZR, ***Wu YZ***. Proteomics-based identification of HSP60 as a tumor-associated antigen in colorectal cancer. ***Proteomics-Clin Appl***, 2007, 1:336.
38. Wang J, Zhang Y, Wei J, Zhang X, Zhang B, Zhu Z, Zou W, Wang Y, Mou Z, Ni B, ***Wu Y***. Lewis x oligosaccharides targeting to DC-SIGN enhanced antigen specific immune response. ***Immunology***. 2007, 121(2):174-82.
39. Xu WY, Wang L, Wang HM, Wang YQ, Liang YF, Zhao TT, ***Wu YZ***. TLR2 and TLR4 agonists synergistically up-regulate SR-A in RAW264.7 through p38. ***Mol Immunol.*** 2007, 44(9):2315-23.
40. Chen Y, Yang C, Jin N, Xie Z, Tang Y, Fei L, Jia Z, ***Wu Y***. Terminal complement complex

C5b-9-treated human monocyte-derived dendritic cells undergo maturation and induce Th1 polarization. ***Eur J Immunol.*** 2007;37(1):167-76.

41. Tang Y, Lin Z, Ni B, Wei J, Han J, Wang H, ***Wu Y***. An altered peptide ligand for naive cytotoxic T lymphocyte epitope of TRP-2(180-188) enhanced immunogenicity. ***Cancer Immunol Immunother.*** 2007;56(3):319-29.

42. Xu Y, Jia Z, Zhou L, Wang L, Li J, Liang Y, Zhao T, Ni B, ***Wu Y***. Evaluation of the safety, immunogenicity and pharmacokinetics of equine anti-SARS-CoV F(ab')2 in macaque. ***Int Immunopharmacol***. 2007 Dec 15; 7(13):1834-40.

43. Luo D, Ni B, Zhao G, Jia Z, Zhou L, Pacal M, Zhang L, Zhang S, Xing L, Lin Z, Wang L, Li J, Liang Y, Shi X, Zhao T, Zou L, ***Wu Y***, Wang X. Protection from Infection with Severe Acute Respiratory Syndrome Coronavirus in a Chinese Hamster Model by Equine Neutralizing F(ab')2. ***Viral Immunology*** 2007 Sep;20(3);495-502

44. Zhu B, Zou L, Cheng X, Lin Z, Duan Y, ***Wu Y***, Zhou F, Chen Z. Administration of MIP-3alpha gene to the tumor following radiation therapy boosts anti-tumor immunity in a murine model of lung carcinoma. ***Immunol Lett.*** 2006;103(2):101-7.

45. Mou Z, Zhou J, Li J, Zou L, Zhou W, ***Wu Y***. Expression of small hairpin RNAs for S100A9 used in the protein function research. ***Leuk Res.*** 2006;30(8):1013-7.

46. Li JT, Fei L, Mou ZR, Wei J, Tang Y, He HY, Wang L, ***Wu YZ***. Immunogenicity of a plant-derived edible rotavirus subunit vaccine transformed over fifty generations. ***Virology.*** 2006;356(1-2):171-8.

47. Wei J, Li JT, Zhang XP, Tang Y, Wang JX, Zhang B, ***Wu YZ***. Identification of an HLA-A*0201-restricted cytotoxic T-lymphocyte epitope in rotavirus VP6 protein. ***Journal of General Virology.*** 2006;87(Pt 11):3393-6.

48. Zhang J, Chen Y, Li J, Zhang R, Wu Y, Zou L, Zhao T, Zhang X, Han J, Chen A, ***Wu Y***. Renal tubular epithelial expression of the coinhibitory molecule B7-DC (programmed death-1 ligand). ***Journal of Nephrology.*** 2006;19(4):429-38.

49. Chen Y, Yang C, Xie Z, Zou L, Ruan Z, Zhang X, Tang Y, Fei L, Jia Z, ***Wu Y***. Expression of the novel co-stimulatory molecule B7-H4 by renal tubular epithelial cells. ***Kidney Int.*** 2006;70(12):2092-9.

50. Han JF, Zhao TT, Liu HL, Lin ZH, Wang HM, Ruan ZH, Zou LY, ***Wu YZ***. Identification of a new HLA-A*0201-restricted cytotoxic T lymphocyte epitope from CML28. ***Cancer Immunol Immunother.*** 2006;55(12):1575-83.

51. Luo D, Ni B, Li P, Shi W, Zhang S, Han Y, Mao L, He Y, ***Wu Y***, Wang X. Protective immunity elicited by a divalent DNA vaccine encoding both the L7/L12 and Omp16 genes of Brucella abortus in BALB/c mice. ***Infect Immun.*** 2006;74(5): 2734-41.

52. Chen Y, Zhang J, Li J, Zhao T, Zou L, Tang Y, Zhang X, ***Wu Y***. Triptolide inhibits B7-H1 expression on proinflammatory factor activated renal tubular epithelial cells by decreasing NF-kappaB transcription. ***Mol Immunol.*** 2006;43(8):1088-98.

53. Chen Y, Zhang J, Li J, Zou L, Zhao T, Tang Y, ***Wu Y***. Expression of B7-H1 in inflammatory renal tubular epithelial cells. ***Nephron Exp Nephrol.*** 2006;102(3-4):e81-92.
54. Wang X, Ni B, Du X, Zhao G, Gao W, Shi X, Zhang S, Zhang L, Wang D, Luo D, Xing L, Jiang H, Li W, Jiang M, Mao L, He Y, Xiao Y, ***Wu Y***. Protection of mammalian cells from severe acute respiratory syndrome coronavirus infection by equine neutralizing antibody. ***Antivir Ther.*** 2005;10(5):681-90.
55. Ni B, Shi X, Li Y, Gao W, Wang X, ***Wu Y***. Inhibition of replication and infection of severe acute respiratory syndrome-associated coronavirus with plasmid-mediated interference RNA. ***Antivir Ther.*** 2005;10(4):527-33.
56. Jia ZC, Zou LY, Ni B, Wan Y, Zhou W, Lv YB, Geng M, ***Wu YZ***. Effective induction of antitumor immunity by immunization with plasmid DNA encoding TRP-2 plus neutralization of TGF-beta. ***Cancer Immunol Immunother.*** 2005;54(5):446-52.
57. Ni B, Gao W, Zhu B, Lin Z, Jia Z, Zhou W, Zhao J, Wang L, ***Wu Y***. Induction of specific human primary immune response to a Semliki forest virus-based tumor vaccine in a trimera mouse model. ***Cancer Immunol Immunother.*** 2005;54(5):489-98.
58. Chen A, Wang L, Zhang J, Zou L, Jia Z, Zhou W, Wan Y, ***Wu Y***. H-2 Kd-restricted hepatitis B virus-derived epitope whose specific CD8+ T lymphocytes can produce gamma interferon without cytotoxicity. ***J Virol.*** 2005;79(9):5568-76.
59. Wan Y, ***Wu Y***, Zhou J, Zou L, Liang Y, Zhao J, Jia Z, Engberg J, Bian J, Zhou W. Cross-presentation of phage particle antigen in MHC class II and endoplasmic reticulum marker-positive compartments. ***Eur J Immunol.*** 2005;35(7):2041-50.
60. Liang Y, Wan Y, Qiu LW, Zhou J, Ni B, Guo B, Zou Q, Zou L, Zhou W, Jia Z, Che XY, ***Wu Y***. Comprehensive antibody epitope mapping of the nucleocapsid protein of severe acute respiratory syndrome (SARS) coronavirus: insight into the humoral immunity of SARS. ***Clinical Chemistry.*** 2005;51(8):1382-96.
61. Zou L, ***Wu Y***, Pei L, Zhong D, Gen M, Zhao T, Wu J, Ni B, Mou Z, Han J, Chen Y, Zhi Y. Identification of leukemia-associated antigens in chronic myeloid leukemia by proteomic analysis. ***Leuk Res.*** 2005;29(12):1387-91.
62. Chen Y, Li J, Zhang J, Zhao T, Zou L, Tang Y, Zhang X, ***Wu Y***. Sinomenine inhibits B7-H1 and B7-DC expression on human renal tubular epithelial cells. ***Int Immunopharmacol.*** 2005;5(9):1446-57.
63. ***Wu Y***, Mou Z, Li J, Zhou W, Wei B, Zou L. Identification of a S100 calcium-binding protein expressed in HL-60 cells treated with all-trans retinoic acid by two-dimensional electrophoresis and mass spectrometry. ***Leuk Res.***2004;28(2):203-7.
64. ***Wu Y***, Zhang J, Chen S, Chen A, Wang L, Li J, Zhao T, Zou L, Tang Y, Tingrong L, Wang F. Frequencies of epitope-specific cytotoxic T lymphocytes in active chronic viral hepatitis B infection by using MHC class I peptide tetramers. ***Immunol Lett.*** 2004;92(3):253-8.
65. Xu GL, Zhu XH, Guo B, ***Wu YZ***. Involvement of CTLA-4 in T-cell anergy induced by

staphylococcal enterotoxin A in vitro. ***Mol Immunol.*** 2004;41(1):1-8.

66. Mou ZR, Li JT, ***Wu YZ***. Altered expression of S100 calcium binding protein in HL-60 cells treated with retinoic acid. ***Molecular & Cellular Proteomics.*** 2004;3(10):55.

67. Ni B, ***Wu Y***. Primary human immunity in a trimera mouse model elicited by recombinant SFV-based tumor vaccine. ***Immunology.*** 2004;361-368.

68. Wang L, ***Wu YZ***, Chen A, Zhang JB, Yang Z, Niu W, Geng M, Ni B, Zhou W, Zou LY, Jiang M. MHC class I-associated presentation of exogenous peptides is not only enhanced but also prolonged by linking with a C-terminal Lys-Asp-Glu-Leu endoplasmic reticulum retrieval signal. ***Eur. J. Immunol.*** 2004;34(12):3582-94.

69. Ni B, Lin Z, Zhou L, Wang L, Jia Z, Zhou W, Diciommo DP, Zhao J, Bremner R, ***Wu Y***. Induction of P815 tumor immunity by DNA-based recombinant Semliki Forest virus or replicon DNA expressing the P1A gene. ***Cancer Detect Prev.*** 2004;28(6):418-25.

70. Lin ZH, ***Wu YZ***, Zhu B, Ni B, Wang L. Toward the Quantitative Prediction of T-Cell Epitopes: QSAR Studies on Peptides Having Affinity with the Class I MHC Molecular HLA-A*0201. ***Journal of Computational Biology.*** 2004;11(4): 683-94.

71. Zhihua L, ***Yuzhang W***, Bo Z, Bing N, Li W. Toward the quantitative prediction of T-cell epitopes: QSAR studies on peptides having affinity with the class I MHC molecular HLA-A*0201. ***J Comput Biol.*** 2004;11(4):683-94

72. ***Wu Y***, Zhang J, Chen S, Chen A, Wang L, Li J, Zhao T, Zou L, Tang Y, Tingrong L, Wang F. Frequencies of epitope-specific cytotoxic T lymphocytes in active chronic viral hepatitis B infection by using MHC class I peptide tetramers. ***Immunol Lett*** 2004 Apr. 15;92(3):253-8

73. Lin ZH, ***Wu YZ***, Wei YL, Ni B, Zhu B, Wang L. A Rapid Method for Quantitative Prediction of High Affinity CTL Epitopes: QSAR Studies on Peptides Having Affinity with the Class I MHC Molecule HLA-A*0201. ***Letters in Peptide Science.*** 2003;10(1):15-23.

74. ***Wu YZ***, Li JT, Mou ZR, Fei L, Ni B, Geng M, Jia ZC, Zhou W, Zou LY, Tang Y. Oral immunization with rotavirus VP7 expressed in transgenic potatoes induced high titers of mucosal neutralizing IgA. ***Virology.*** 2003;313(2):337-42.

75. Zhu B, Chen Z, Cheng X, Lin Z, Guo J, Jia Z, Zou L, Wang Z, Hu Y, Wang D, ***Wu Y***. Identification of HLA-A*0201-restricted cytotoxic T lymphocyte epitope from TRAG-3 antigen. ***Clin Cancer Res.*** 2003;9(5):1850-7.

76. Liu HL, ***Wu YZ***, Zhao JP, Ni B, Jia ZC, Zhou W, Zou LY. Effective elicitation of anti-tumor immunity by collocation of antigen with encoding gene in the same vaccine. ***Immunol Lett.*** 2003;89(2-3):167-73.

77. ***Wu YZ***, Zhao TT, Ni B, Zou LY, Liu HL, Zhu B. Expression of TRAG-3 in breast cancer. ***Int.J.Cancer*** 2003;107(1):167-8.

78. Geng M, ***Wu YZ***, Lin ZH, Jia ZC, Zhou W, Zou LY. Screening of HLA-A2 restricted epitopes using molecular simulation. ***Chinese Science Bulletin***. 2003;48(18):1962-6.

79. Zhu B, Chen Z, Cheng X, ***Wu Y***. Induction of TRAG-3 expression in A549 lung

adenocarcinoma cell line by 5-aza-2' deoxyazacytidine. ***Lung Cancer.*** 2002;38(3):321-2.

80. Lin ZH, Wu YZ, Quan XJ, Zhou YG, Ni B, Wan Y. Use of a novel electrotopological descriptor for the prediction of biological activity of peptide analogues. ***Letters in Peptide Science.*** 2002;9(6):273–81.

81. Chen Z, Zhu B, ***Wu Y***. Expression of TRAG-3 antigen in non-small-cell lung carcinomas. ***Lung Cancer.*** 2002;38(1):101-2.

82. ***Wu YZ***, Li YQ, Lin Y. Expression of MAGE-12 in Lung Cancer. ***Lung Cancer*** 2002;35(1):95.

83. ***Wu YZ***, Zhao JP, Wan Y, Jia ZC, Zhou W, Bian J, Ni B, Zou LY, Tang Y. Mimovirus: a novel form of vaccine that induces hepatitis B virus-specific cytotoxic Tlymphocyte responses in vivo. ***J Virol.***2002;76(20):10264-9.

84. ***Wu Y***, Wan Y, Bian J, Zhao J, Jia Z, Zhou L, Zhou W, Tan Y. Phage display particles expressing tumor-specific antigens induce preventive and therapeutic anti-tumor immunity in murine p815 model. ***Int.J.Cancer*** 2002;98(5):748-53.

85. Guan XJ, Guan XJ, ***Wu YZ***, Jia ZC, Shi TD, Tang Y. Construction and characterization of an experimental ISCOMS-based hepatitis B polypeptide vaccine. ***World J Gastroenterol.*** 2002 Apr;8(2):294-7.

86. ***Wu YZ***, Wan Y, Li DG, Yan Tang Y, Zhou W. A mimotope of Pre-S2 region of surface antigen of viral hepatitis B screened by phage display. ***Cell Research.*** 2001;11(3):203-8.

87. Wan Y, ***Wu Y***, Bian J, Wang XZ, Zhou W, Jia ZC, Tan Y, Zhou L. Induction of hepatitis B virus-specific cytotoxic T lymphocytes response in vivo by filamentous phage display vaccine. ***Vaccine.*** 2001;19(20-22):2918-23.

88. Hu WG, Zhu XH, ***Wu YZ***, Jia ZC. Localization of a T-cell epitope of superantigen toxic shock syndrome toxin 1 to residues 125 to 158. ***Infection and Immunity.*** 1998;66(10):4971-5.

89. ***Wu YZ***, Zhu XH. A new approach for B-cell epitope prediction in viral protein. ***Chinese Science Bulletin.*** 1995:40(9):761-6.

90. Shi TD, ***Wu YZ***. Molecular design and immunological investigations on the therapeutic peptide vaccines against viral hepatitis B. ***The Immunologist.*** 1998;S:330-4.

91. ***Wu YZ***, Shi TD. EBVD-a new approach to vaccine design. ***The Immunologist.*** 1998;S:315-8.